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(54) Title: SURFACTANT FOR USE IN EXTERNAL PREPARATIONS FOR SKIN AND EXTERNAL PREPARATION FOR SKIN CONTAINING THE SAME

(57) Abstract

Disclosed are a surfactant for use in external preparations for skin including a lipopeptide compound derived from prokaryotes and having low skin penetration and low irritation to the skin, an external preparation for skin containing such a surfactant, such as a cosmetic, and an external preparation for skin, such as a transparent cosmetic, e.g., a transparent cosmetic containing such a surfactant and a sequestering agent.

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DESCRIPTION

Surfactant for use in external preparations for skin and external preparation for skin containing the same

TECHNICAL FILED

The present invention relates to a surfactant for use in external preparations for skin and to an external preparation for skin containing it. More particularly, the present invention relates to a surfactant for use in external preparations for skin having low skin permeability (penetrability) and low irritation, an external preparation for skin, such as cosmetic, containing such a surfactant, and an external preparation for skin, such as a transparent cosmetic, containing such a surfactant and a sequestering agent.

BACKGROUND ART

Hitherto, in external preparations for skin, such as cosmetics, there have been used anionic surfactants composed of aliphatic higher alcohol sulfates, aliphatic higher alcohol phosphates, N-long chain acyl glutaminates, etc., ether type nonionic surfactants, e.g., aliphatic higher alcohol ethylene oxide adducts, nonionic surfactants composed of higher fatty acid and polyhydric alcohols, as emulsifiers, dispersants, solubilizers, etc.

However, skin irritation of these surfactants cannot be said to be sufficiently low to individuals who have allergic constitution, suffering, e.g., pollenosis, atopic dermatitis, etc. so that external preparations for skin containing such have insufficient safety to skin and improvement thereof has been desired.

Further, even if external preparations for skin are prepared using surfactants having sufficiently low irritation to the skin, the external preparations for skin contain in addition to surfactants irritating substances such as salicylic acid, paraben or hexachlorophene as an antiseptic. Therefore, in order to reduce their irritation to the skin, development of low irritation external preparations for skin has been desired.

Known examples of low irritation surfactants include amino acid derivatives. For example, there have been proposed basic amino acid derivatives produced by reacting a glycidyl ether and a basic amino acid (Japanese Patent Application Laid-open No. Hei 9-271655 (European Patent Application Laid-open No.788,832(A1)), and certain water-soluble glycoxide type surfactants as a surfactant having low irritation and alleviating the irritation of other skin-irritating substances (Japanese Patent Application Laid-open No. Hei 9-235587).

Further, with view to prevent glycine derivatives from coloring and deterioration, there have been proposed detergent compositions having blended therein a metal chelating agent and an antioxidant (Japanese Patent Application Laid-open Nos. Hei 9-78085, Hei 9-87673, and Hei 10-237488). However, these surfactants have problems that they are not fully satisfactory in low irritation, they have low effect of reducing irritation by irritating substances other than surfactants, they decrease the surface activity of other surfactants, their deterioration cannot be prevented completely, and so on.

DISCLOSURE OF THE INVENTION

Therefore, an object of the present invention is to provide a surfactant for use in external preparations for skin, having low irritation to the skin.

Another object of the present invention is to provide a surfactant for use in external preparations for skin, not only having low skin irritation to the skin itself but also reducing the irritation of skin-irritating substances.

Further, an object of the present invention is to provide an external preparation for skin, such as a cosmetic, containing the above-described surfactant for use in external preparations for skin.

Furthermore, an object of the present invention is to provide an external preparation for skin containing the above-described surfactant for use in external preparations for skin, retaining transparency for cosmetics required of transparency.

The present inventors have made intensive research with view to achieving the above-described objects and as a result they have found that when used as a surfactant, a compound produced by fermentation by prokaryotes such as *Bacillus* microbes is low in skin penetrability, is low in irritation to the skin and, surprisingly, has an effect of reducing the irritation by skin-irritating substances.

Further, the present inventors have found that existence of a minute amount of an alkaline earth metal such as calcium or magnesium in external preparations for skin containing the above-described surfactant results in that the surfactant and the alkaline earth metal form a water-insoluble salt, which precipitates and make the preparation turbid whereas blending a sequestering agent together with the surfactant in the external preparation for skin prevents the formation of the water-insoluble salt of the surfactant without affecting the low irritation of the surfactant and the effect of reducing the irritation of skin-irritating

substances, so that transparency of the preparation can be retained.

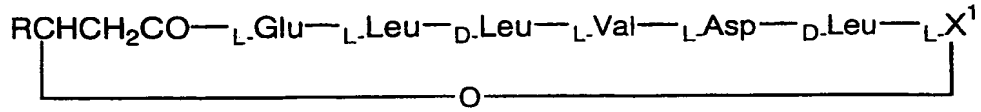
Based on these findings, the present invention provides a surfactant for use in external preparations for skin, an external preparation for skin, and a cosmetic as described below.

[1] A surfactant for use in external preparations for skin comprising a compound derived from a prokaryote.

[2] The surfactant for use in external preparations for skin as described in item 1 above, wherein the prokaryote is a Bacillus microbe.

[3] The surfactant for use in external preparations for skin as described in item 1 above, wherein the compound derived from prokaryote is a lipopeptide compound or its salts.

[4] The surfactant for use in external preparations for skin as described in item 3 above, wherein the lipopeptide compound is at least one compound represented by formula (2) below



(wherein X^1 is an amino acid selected from the group consisting of leucine, isoleucine, valine, glycine, serine, alanine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine,

cystein, methionine, phenylalanine, tyrosine, tryptophan, histidine, proline, 4-hydroxyproline, and homoserine, and R has 9 to 13 carbon atoms and is a n-alkyl group, an isoalkyl group, or an anteisoalkyl group).

[5] The surfactant for use in external preparations for skin as described in item 4 above, wherein X^1 is leucine, isoleucine or valine.

[6] The surfactant for use in external preparations for skin as described in item 3 above, wherein the lipopeptide compound is plipastatin, arthrofactin, iturin, or serrawettin.

[7] The surfactant for use in external preparations for skin as described in any one of items 1 to 6 above, wherein the surfactant has an effect of inhibiting the skin penetration of a skin-irritating substance.

[8] The surfactant for use in external preparations for skin as described in any one of items 1 to 6 above, wherein the surfactant has an effect of inhibiting the skin penetration of a skin-irritating substance and reduces irritation of a skin-irritating substance.

[9] The surfactant for use in external preparations for skin as described in item 8 above, wherein the skin-irritating substance is an antiseptic.

[10] The surfactant for use in external preparations for skin as described in item 9 above, wherein the antiseptic is a paraben compound.

[11] An external preparation for skin comprising a surfactant for use in external preparations as described in any one of items 1 to 10 above.

[12] The external preparation for skin as described in item 11 above, wherein the surfactant for use in external preparations for skin is in a content of 0.01 to 30 wt%.

[13] The external preparation for skin as described in item 11 or 12 above, further comprising a sequestering agent.

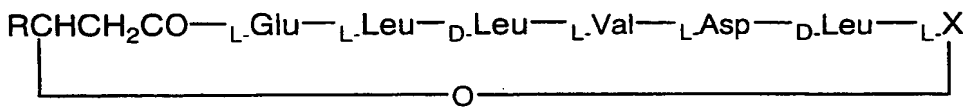
[14] The external preparation for skin as described in item 13 above, wherein the surfactant for use in external preparations for skin is in a content of 0.01 to 30 wt% and the sequestering agent is in a content of 0.0001 to 30 wt%.

[15] A cosmetic comprising an external preparation for skin as described in any one of items 11 to 14 above.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that a lipopeptide compound derived from a prokaryote has a low irritation to the skin and reduces the irritation of skin-irritating substances and has applied this to a surfactant for use in external preparations for skin.

Typical examples of the lipopeptide compound derived from a prokaryote includes surfactin. Surfactin is a compound, which is produced usually by a prokaryote, has a lipopeptide structure represented by the formula 1 below.



wherein X is leucine, isoleucine or valine, R has 9 to 13 carbon atoms and represents a n-alkyl group, an isoalkyl group or an anteisoalkyl group.

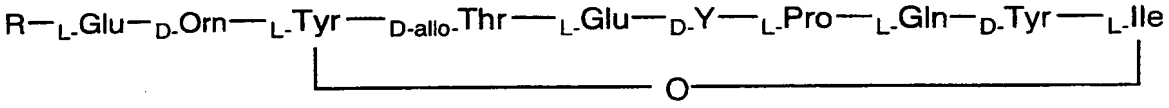
Generally, *Bacillus* microbes are used as the prokaryote. Specific examples thereof include *Bacillus subtilis* IAM 1213 strain, IAM 1069 strain, IAM 1259 strain, IAM 1260 strain, IFO 3035 strain, ATCC 21332 strain, etc.

Surfactin can be obtained without difficulty by cultivating these microbes and purifying lipopeptide compounds that the microbes produced. Purification can be performed, for example, by rendering the culture broth acidic by addition of hydrochloric acid, etc., filtering surfactin which precipitated, dissolving the precipitate in an organic solvent such as methanol, and then optionally practicing ultrafiltration, treatment with activated carbon, crystallization, etc. Precipitation by addition of an acid may be replaced by

precipitation by addition of a calcium salt (Biochem. Bioph. Res. Commun., 31: 488-494 (1968)).

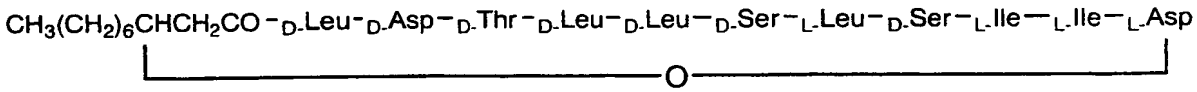
Compounds having a lipopeptide structure derived from prokaryotes, other than surfactin, may be used similarly. Examples of such compounds include plipastatin (J. Antibiot., Vol. 39, No. 6, 745-761, 1986), arthrofactin (J. Bacteriol., Vol. 175, No. 20, 6459-6466, 1993), iturin (Biochemistry, Vol. 17, No. 19, 3992-3996, 1978, serrawettin (J. Bacteriol., Vol. 174, No. 6, 1769-1772, 1992).

Plipastatin

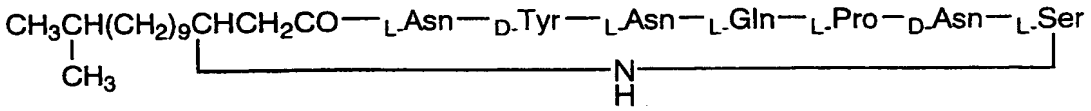


(wherein Y is Val or Ala and Orn stands for ornithine.)

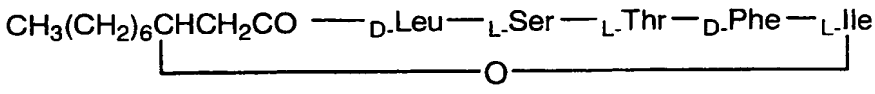
Arthrofactin



Iturin



Serrawettin



Hereafter, the present invention will be described specifically referring to surfactin as a typical example.

Surfactin generally comprises at least one compound represented by the formula 1 above.

In the formula 1, of the groups having 9 to 13 carbon atoms represented by R, the n-alkyl group is a straight chain alkyl group, the isoalkyl group usually has the structure $(CH_3)_2CH-(CH_2)_n-$, and the anteisoalkyl group usually has the structure $CH_3-CH_2-CH(CH_3)-(CH_2)_n-$.

When surfactin is utilized, the culture broth may be used as it is or purified before it can be used.

As will be apparent from the formula 1, surfactin may be used as a metal salt or organic ammonium salt of a carboxyl group derived from the amino acid structural unit. The metal which serves as a counter ion may be any types of metals so far as they form a salt with surfactin, not to speak of alkali metals such as sodium, potassium, and lithium, alkaline earth metals such as calcium and magnesium. The organic ammonium includes trimethylamine, triethylamine, tributylamine, monoethanolamine, diethanolamine, triethanolamine,

lysine, arginine, choline, etc. Among them, preferred are alkali metal salts, particularly sodium salt.

Surfactin shows an anion activity due to the carboxyl group of L-Glu (L-glutamic acid), L-Asp (L-aspartic acid) therein.

The low irritation to the skin of surfactin is considered to be attributable to the fact that surfactin is a cyclic compound of a complexed structure and bulky so that it has a low skin penetrability. Also, surfactin is considered to reduce the irritation of skin-irritating substances because of its masking effect by surrounding the skin-irritating substances.

Further, the present invention provides an external preparation for skin by utilizing surfactin as a surfactant for use in external preparations for skin and blending it together with a sequestering agent to retain transparency.

In the present invention, the surfactant for use in external preparations for skin used has a low skin penetrability and is expected to exhibit the above-described masking effect.

Further, those compounds based on surfactin but with varied amino acid composition, for example, X in the formula 1 is substituted by glycine, serine, alanine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, cystein,

methionine, phenylalanine, tyrosine, tryptophan, histidine, proline, 4-hydroxyproline, and homoserine, the compound of the formula 2 above, may also be used.

Surfactin and the above-described compounds may also be those obtained by other methods, for example, chemical synthesis, as well as those derived from prokaryotes such as *Bacillus* microbes, and can be used similarly.

The sequestering agent used in the present invention will be explained below.

If metal ions exist in transparent external preparations for skin, they cause deterioration of the quality of external preparations for skin, such as generation of turbidity or precipitation. The sequestering agent is used for the purpose of preventing such.

The sequestering agent which can be used in the present invention may be of any type so far as it has an acidic group having a salt-forming ability or an atomic group having an ability of coordination and can sequester metal ions. Specific examples of the sequestering agent includes L-alanine, DL-alanine, trisodium ethylenediaminehydroxyethyltriacetate, trisodium ethylenediaminehydroxyethyltriasetate dihydrate, edetic acid, dipotassium edetate dihydrate, disodium edetate, disodium calcium edetate, trisodium

edetate, tetrasodium edetate, tetrasodium edetate dihydrate, tetrasodium edetate tetrahydrate, sodium citrate, gluconic acid, sodium gluconate, tartaric acid, phytic acid, sodium polyphosphate, sodium metaphosphate, tetrasodium L-glutaminate diacetate, etc.

These may be used singly or two or more of them may be used simultaneously.

Of these, disodium edetate and sodium citrate are particularly preferred.

The sequestering agent forms salts with alkaline earth metals such as calcium and magnesium so that it prevents the formation of salts between surfactin and alkaline earth metals.

The external preparation for skin of the present invention contains the above-described surfactant or the above-described surfactant and sequestering agent. In the external preparations for skin, the surfactant used in the present invention serves as an emulsifier, a dispersant, a solubilizer, a wetting agent, a detergent, a humectant, etc. and also acts as an irritation-reducing agent for skin-irritating substances. There is no limitation on the form in which the surfactant is contained in external preparations for skin, which may be achieved by any one of dissolution, emulsification, dispersion, mixing, etc. and may be in

any form such as liquid, milky lotion, gel, solid (inclusive of powder and granules). It may be in the state where vesicles are formed in a solution.

The amount of surfactant in external preparations for skin is generally in a range of 0.01 to 30 wt%. The amount of sequestering agent is generally equivalent to or larger than the amount of the alkaline earth metal contained in the external preparation for skin, which gives sufficient effects. More specifically, it may be used in a range of 0.0001 to 3 wt%, preferably 0.001 to 0.2 wt%.

A typical example of external preparations for skin is a cosmetic. Specific examples thereof include skin milk, skin cream, foundation cream, massaging cream, cleansing cream, shaving cream, cleansing foam, a beauty wash, lotion, pack, shampoo, rinse, a hair restoration agent, a hair tonic, a hair dye, a hair dressing, dentifrice, a gargle, permanent waving agent, ointment, bath additive, body soap, etc. Any type of external preparations for skin may be included so far as it is brought in contact with the skin when in use. Sex and age of users are irrelevant.

The skin-irritating substances whose irritation is reduced by the surfactant for use in external preparations for skin according to the present invention includes antiseptics, ultraviolet absorbents,

antioxidants, dyes, beautifying and whitening agents, hair dyes, perfumes, alcohols, metal soaps, surfactants other than those of the invention, and so on.

Hereafter, specific examples thereof will be described. In particular, the surfactant of the present invention is effective in reducing the irritation of paraben compounds, which are antiseptics.

Skin-irritating substances:

Bacteriocidal antiseptics such as salicylic acid, paraben compounds (methylparaben, propylparaben, butylparaben, ethylparaben, etc.), hexachlorophene, imidazolidinylurea, quaternium-15, DM hydantoin, phenoxyethanol, and benzalkonium salts.

Sun screening agents such as p-aminobenzoic acid, antioxidants such as dibutylhydroxyltoluene, butylhydroxyanisole and alkyl gallates, para-amino acids such as 2-hydroxy-4-methoxybenzophenone, octyldimethyl-p-aminobenzoate, and ethylhexyl-p-methoxycinnamate, organic ultraviolet absorbents such as hydroxybenzophenone-base, benzofuran-base, salicylic acid-base, coumarin-base, azole-base ultraviolet absorbents.

Ultraviolet rays reflection scattering agent such as titanium oxide, kaolin, and talc.

Vitamin agents such as vitamin A, C, E.

Pigments such as talc, kaolin, calcium carbonate, magnesium carbonate, magnesium silicate, silicic anhydride, titanium oxide, zinc oxide, red iron oxide, yellow iron oxide, chromium oxide, chromium hydroxide, carbon black, ultramarine, bismuth oxychloride, mica titanium mineral, and mica and organic and tar-base dyes such as butter yellow.

Beautifying and whitening agents such as kojic acid, albutin, mulberry root bark, placenta extract, SS albutin, ellagic acid, chamomile extract, and ascorbic acid derivatives.

Hair dyes such as oxidative dyes and acidic dyes and color aids, e.g., 5-amino-o-cresol, 2-amino-4-nitrophenol, 2-amino-5-nitrophenol, 1-amino-4-methylaminoanthraquinone, 3,3'-iminodiphenol, 2,4-diaminophenoxyethanol hydrochloride, 2,4-diaminophenol hydrochloride, toluene-2,5-diamine hydrochloride, nitro-p-phenylenediamine hydrochloride, p-phenylenediamine hydrochloride, N-phenyl-p-phenylenediamine hydrochloride, m-phenylenediamine hydrochloride, o-aminophenol, catechol, N-phenyl-p-phenylenediamine acetate, 1,4-diaminoanthraquinone, 2,6-diaminopyridine, 1,5-dihydroxynaphthalene, diphenylamine, ammonium carbonate, toluene-2,5-diamine, toluene-3,4-diamine, α-naphthol, nitro-p-phenylenediamine, p-aminophenol,

p-nitro-o-phenylenediamine, p-phenylenediamine, p-methylaminophenol, picramic acid, sodium picramate, N,N-bis(4-aminophenyl)-2,5-diamino-1,4-quinone-diimine, 5-(2-hydroxyethylamino)-2-methylphenol, sodium 2-hdryoxy-5-nitro-2',4'-diaminoazobenzene-5-sulfonate, hydroquinone, pyrogallol, N-phenyl-p-phenylenediamine, phloroglucin, hematein, gallic acid, m-aminophenol, m-phenylenediamine, 5-amino-o-cresol sulfate, 2-amino-5-nitrophenol sulfate, o-aminophenol sulfate, o-chloro-p-phenylenediamine sulfate, 4,4'-diaminodiphenylamine sulfate, 2,4-diamminophenol sulfate, toluene-2,5-diamine sulfate, nitro-p-phenylenediamine sulfate, p-aminophenol sulfate, p-nitro-o-phenylenediamine sulfate, p-nitro-m-phenylenediamine sulfate, p-phenylenediamine sulfate, p-methylaminophenol sulfate, m-aminophenol sulfate, m-phenylenediamine sulfate, etc.

Perfumes such as sesquiterpene alcohol, geraniol, and linalool.

pH adjusting agent, buffer, or chelating agent, e.g., disodium edetate, ethylenediaminetetraacetic acid salts, pyrophosphoric acid salts, hexametaphosphoric acid salts, tartaric acid, gluconic acid, sodium hydroxide, triethanolamine, citric acid, sodium citrate, boric acid, borax, potassium hydrogen phosphate, etc.

Astringent such as zinc p-phenolsulfonate.

Alcohols such as ethanol and isopropanol.

Metal soaps such as magnesium, calcium and aluminum stearates, zinc laurate, and zinc palmitate.

In the external preparation for skin of the present invention, known synthetic surfactant may be used in combination and also the irritation of the surfactant can be reduced.

The surfactant in this case include, for example, nonionic surfactants such as oleophilic glycerin monostearate, self-emulsifying glycerin monostearate, polyglycerin monostearate, sorbitan monooleate, polyethylene glycol monostearate, polyoxyethylene sorbitan monooleate, polyoxyethylene cetyl ether, polyoxyethylenated sterol, polyoxyethylene alkyl ether, polyoxyethylene fatty acid ester, polyoxyethylene sorbitan fatty acid ester, polyoxyethylenated lanolin, poloxyethylenated beeswax, and polyoxyethylene-hardened castor oil, anionic surfactants such as sodium stearylphosphate, potassium palmitate, sodium cetylsulfate, sodium laurylphosphate, triethanolamine palmitate, sodium polyoxyethylene laurylphosphate and sodium N-acylglutaminate, cationic surfactants such as stearyldimethylbenzylammonium chloride and stearyltrimethylammonium chloride, amphoteric

surfactants such as alkylaminoethylglycine hydrochloride liquid, and lecithin.

As other irritating substances, among oils and fats, oxidized lipids and lipid peroxides may be irritants. For example, it is when lipids as set forth below are oxidized.

There can be cited, for example, plant oils and fats such as castor oil, olive oil, cacao oil, camellia oil, coconut oil, haze wax, jojoba oil, grape seed oil, avogado oil, beefsteak plant oil, perilla oil, animal oils and fats such as mink oil, yolk oil, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), waxes such as beeswax, spermaceti, lanolin, carnauba wax, and candelilla wax, hydrocarbons such as liquid paraffin, squalane, microcrystalline wax, ceresin wax, paraffin wax, and vaseline, natural and synthetic fatty acids such as lauric acid, myristic acid, stearic acid, oleic acid, isostearic acid, and behenic acid, natural and synthetic higher alcohols such as cetanol, stearyl alcohol, hexyldecanol, octyldodecanol, and lauryl alcohol, esters such as isopropyl myristate, isopropyl palmitate, isopropyl adipate, octyldodecyl myristate, octyldodecyl oleate, and cholesterol oleate.

Also, permanent waving agent such as thioglycollic acid includes irritants.

In addition, there can be cited those substances which are converted into irritants during storage or use by physical, chemical or biological effects, for example, peroxides or various irritating decomposates. However, this invention is not limited thereto.

In addition to the above-described skin-irritating substances, the external preparation for skin of the present invention may contain usually used components such as surfactants, humectants, thickening agent, antiphlogistics, plant extract components, and other components as described below.

Examples of surfactant include sodium monofluorophosphate, fatty acid salts, alkylbenzenesulfonates, alkylnaphthalenesulfonates, alkylsulfonates, α-olefinsulfonates, dialkylsulfosuccinates, α-sulfonated fatty acid salts, alkylsulfates, polyoxyethylene alkyl ether sulfates, polyoxyethylene alkyl phenyl ether sulfates, polyoxyethylene styrenated phenyl ether sulfates, alkylphosphates, polyoxyethylene alkyl ether phosphates, polyoxyethylene alkyl phenyl ether phosphate, naphthalenesulfonate formalin condensate, polyoxyethylene alkyl ether, polyoxyethylene alkyl phenyl ether, polyoxyethylene polystyryl phenyl ether, polyoxyethylene polyoxypropylene glycol, polyoxyethylene polyoxypropylene alkyl ether,

polyhydric alcohol fatty acid partial ester, polyoxyethylene polyhydric alcohol fatty acid partial ester, polyoxyethylene fatty acid ester, polyglycerin fatty acid ester, polyoxyethylenated castor oil, fatty acid diethanolamide, polyoxyethylene alkylamine, triethanolamine fatty acid partial ester, trialkylamine oxide, fatty acid amine salt, tetraalkylammonium salt, trialklbenzylammonium salt, alkylpyridinium salt, 2-alkyl-1-alkyl-1-hydroxyethylimidazolium salt, N,N-dialkylmorpholinium salt, polyethylene polyamine fatty acid amide salt, etc.

Examples of humectant includes polyhydric alcohols, such as glycerin, propylene glycol, 1,3-butylene glycol, sorbitol, polyglycerin, polyethylene glycol, and dipropylene glycol, natural moisturizing factor (NMF) components such as amino acids and sodium lactate, water-soluble polymers such as collagen, mucopolysaccharide and chondroitinsulfate, moisture-conditioner/humectants such as maltitol, sodium pyrolidonecarboxylate, polyoxyethylene methyl glucoside, hyaluronic acid, hyaluronic acid derivatives, ceramide, ceramide derivatives, ceramide analogues, and glucose.

Examples of thickening agent include natural polymers such as sodium alginate, xanthan gum, quince seed gum, tran gum, bee gum, pectin, alginates,

laponite, aluminum silicate, quince seed extract, tragacanth gum, and starch, semi-synthetic polymers such as methylcellulose, hydroxyethylcellulose, carboxymethylcellulose, soluble starch, and cationated cellulose, synthetic polymers such as acrylic polymer and polyvinyl alcohol, etc.

Antiphlogistics include salicylic acid derivative type antiphlogistics, aniline derivative type antiphlogistics, spasmolysants, pyrazolone derivative type antiphlogistics, indomethacin antiphlogistics, mephenamic acid antiphlogistics, anti-histamin agent, anti-allergy agent, anti-inflammatory enzyme agent, steroid agent, glycyrrhizin, azulene, allantoin, etc.

Combined use of these antiphlogistics will promote antiphlogistic effect on wound. Their usage may be generally on the order of 0.001 to 10 mol/l (external preparation for skin).

Plant extract components include triclosan (Irgasan-DP300 available from Ciba-Geigy Co., Ltd.), glycyrrhizic acid or its sodium or potassium salt or other salts, triethanolamine, hinoki extract, hinokithiol, edetates, propylene glycol, beefsteak plant extract, rosemary extract, rose extract, chamomile extract, Melissa extract, sage extract, licorice extract, jojoba extract, N-acyl-L-glutamic acid or its sodium salt or other salts, cetanol,

Sappiness mukorossi extract, squalanes such as plant squalane, etc.

Other components include nutrients such as amino acids and amino acid derivatives, emolients such as ester oils and higher alcohols, abrasives such as calcium phosphate, aluminum hydroxide, calcium pyrophosphate, and insoluble sodium metaphosphate, ultraviolet absorbents, ultraviolet scattering agents, and those components which are described in the following raw material lists (1) to (8).

(1) Cosmetics raw material nonstandardized components standard (Yakuji Nippo, published October 14, 1993, pages 39-1368)

(2) Japan general use cosmetics raw material list, 2nd ed. (Yakuji Nippo, published March 25, 1989, pages 1-509)

(3) Japanese general use cosmetics raw material list, 3rd ed. (Yakuji Nippo, published June 30, 1994, pages 1-612)

(4) Medical drugs Japanese medicines, 21st ed. (Yakuji Nippo, published 1997, pages 1-2100)

(5) General drugs Japanese medicines, 1998-99 (Yakuji Nippo, November 10, 1997)

(6) 13th Revised Japan Pharmacopoeia First Supplement (Yakugyo Jiho, published January 31, 1998, pages 58-190)

(7) List of laws and ordinances relating to existing additives register associated with amendment of food hygiene law (edited by Food chemistry section, life hygiene bureau, Ministry of Commonwealth, published July 10, 1996, Social Insurance Publishers, pages 5-221)

(8) List of standards on the components of food additives, 8th ed. (Japan Science Feeding Stuff Association, published November 18, 1996, pages 7-827).

The surfactants, humectants, thickening agents, antiphlogistics, plant extracts components and other blending components may be added solely or in combination. There is no limitation on the addition amount thereof but usually they may be added in preparations in amounts in a range of 0.0001 to 80 wt%.

BEST MODE FOR CARRYING OUT THE INVENTION

Next, the present invention will be described in further detail by examples and formulations. However, the present invention should not be construed as being limited thereto. In the following examples, all "%" means "wt%" unless other indicated specifically as in the case of toxicity, for example.

Production Example

Bacillus subtilis ATCC 21332 strain was inoculated in a medium (1% polypeptone, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, adjusted with NaOH to pH 7, balance water) and incubated at 35℃ at 160 rpm for 12 hours. 100 ml of the culture medium was inoculated into a 2-liter fermenter charged with a medium containing soybean powder and maltose as main components and potassium hydrogen phosphate, magnesium sulfate, calcium salt, iron salt, and manganese salt as inorganic salts, and incubated at 35℃ with stirring and strong aeration for 48 hours. During the incubation, caustic soda was added to maintain the medium at pH 7.0 to 7.5. After completion of the incubation, the medium was centrifuged to remove the bacteria cells and the resultant culture supernatant was collected. A portion of the culture supernatant was freeze-dried to obtain a dried medium preparation. The remaining culture supernatant was adjusted with hydrochloric acid to pH 2 to precipitate a surfactin fraction. The supernatant was removed by centrifugation and the surfactin fraction was dissolved in an acetone organic solvent. The resultant solution was passed through ultrafiltration membrane resistant to organic solvents (Cefilt UF10,000, a ceramic membrane filter manufactured by Nippon Gaishi) to recover a liquid fraction, thereby removing high molecular weight

impurities. Then, to the liquid fraction was added activated carbon (ϕ 20μm) to deodorize and decolorize it. Thereafter, the activated carbon was removed by filtration and the filtrate was concentrated to dryness in an evaporator. Then, the resulting solids were dissolved in water while adding caustic soda thereto to maintain the pH around 7. The resultant solution was freeze-dried to obtain purified surfactin sodium salt powder. The dried medium preparation and surfactin sodium salt were used in the following tests.

Surface tension tests

The dried medium preparation and purified surfactin sodium salt were each dissolved in water in an amount of 0.1 wt% and their ability of decreasing surface tension was tested. Surface tension was measured by a plate method (25℃) using an automatic surface tension meter CBVP-Z type manufactured by Kyowa Kaimen Kagaku Co., Ltd. Table 1 shows the results.

Table 1: Surface Tension

Water	72.1 mN/m
Aqueous solution of dried medium preparation (0.1 wt%)	28.4 mN/m
Aqueous solution of purified surfactin sodium (0.1 wt%)	27.6 mN/m

Example 1: Skin irritation test of surfactant

Using a three-dimensional skin model (trade name: Three-dimensional cultured skin model, manufactured by Gunze), skin irritation tests were performed. As the test substances were used the surfactin sodium salt and dried medium preparation of the Production Example above, and SDS (sodium dodecylsulfate), Amisoft LS-11 (manufactured by Ajinomoto, hereafter, referred to as Amisoft). The test substances were adjusted with PBS (Phosphate Buffer Saline, pH 7) so that they were in various concentrations. To the thus-adjusted test substances was exposed the skin model for 1 hour. Thereafter, the test substances were washed and incubated for 16 hours in a medium attached to the above-described three-dimensional skin model. After the incubation, a solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added and pigments were extracted with isopropanol rendered acidic with hydrochloric acid, followed by measurement of absorbance at a wavelength of 570 nm. This value was called A. Also, as a control, similar operations were repeated without addition of test substances and absorbance at a wavelength of 570 nm was measured. This value was called B. The MTT solution was added to PBS and extraction was performed similarly. Absorbance of the extract measured at a

wavelength of 570 nm was named C. From these values was calculated cytotoxicity. Calculation was made according to the following equation.

Cytotoxicity (%) = (1-(A-c)/(B-C)) x 100

Plotting the concentration of a test substance on the axis of abscissa and cytotoxicity on the axis of ordinate, a graph was obtained, from which the concentration of a test compound at 50% cytotoxicity was read. Whether this value is large or small indicates whether skin irritation is strong or weak. Table 2 shows concentrations at 50% cytotoxicity.

Table 2

Surfactant	Concentration of surfactant at 50% cytotoxicity
Surfactin sodium salt	24.4 %
Dried medium preparation	32.8 %
Amisoft	2.7 %
SDS	0.2 %

As shown in Table 2, it is apparent that the surfactin sodium salt and dried medium preparation of the inventive product exhibited very low skin irritation as compared with Amisoft and SDS, respectively.

Example 2: Skin penetration test of skin-irritating substances

One (donor side) of two chambers separated by a hairless mouse skin was filled with a phosphate buffer (pH 7) having dissolved therein a substance having the composition described in Table 3 and the other (receiver side) was filled with a phosphate buffer solution (pH 7). After 5 hours, the concentration of methylparaben on the receiver side was measured. Table 3 shows the results. As will be apparent from the results, surfactin suppressed the skin penetration of skin-irritating substances.

Table 3

Composition on donor side	Concentration of methylparaben of receiver
0.1% Methylparaben	5.7 ppm
0.1% Methylparaben + 1% Surfactin sodium salt	3.6 ppm
0.1% Methylparaben + 1% dried medium preparation	3.8 ppm
0.1% Methylparaben + 1% SDS	23.0 ppm

Example 3: Skin irritation tests with external preparation for skin - 1

Milky lotions having the respective compositions shown in Table 4 were prepared by a conventional method and were evaluated for their skin irritation. In the same manner as in Example 1, a skin model was exposed to each of the prepared test substances (milky lotions described in Table 4) for 1 hour. Thereafter, the test

substance was washed and incubated in the above-described medium for 16 hours. After the incubation, a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added, and pigments were extracted with isopropanol rendered acidic with hydrochloric acid, followed by measurement of absorbance and calculation of cytotoxicity. The cytotoxicity was calculated according to the equation described in Example 1.

Table 5 shows the cytotoxicity of each test substance.

Table 4

Component(%)	Inventive preparation		Comparative preparation	
	1	2	1	2
Surfactin sodium salt	3.0	—	—	—
Dried medium preparation	—	3.0	—	—
Amisoft	—	—	3.0	—
SDS	—	—	—	3.0
Avogado oil	11.0	11.0	11.0	11.0
Behenyl alcohol	0.6	0.6	0.6	0.6
Stearic acid	0.4	0.4	0.4	0.4
1,3-Butylene glycol	10.1	10.1	10.1	10.1
Perfume	0.4	0.4	0.4	0.4
Purified water	Balance	Balance	Balance	Balance

Table 5

Test Substance	Cytotoxicity
Inventive preparation 1	0.0%
Inventive preparation 2	0.0%
Comparative preparation 1	72.5%
Comparative preparation 2	98.5%

As will be apparent from the results in Table 5, the inventive preparation had very low irritation to the skin.

Example 4: Skin irritation tests with external preparation for skin - 2

One (donor side) of two chambers separated by a hairless mouse skin was filled with a cosmetic having the composition described in Table 6 and the other (receiver side) was filled with a phosphate buffer solution (pH 7). After 5 hours, the concentration of methylparaben on the receiver side was measured. Table 7 shows the results.

As will be apparent from the results, the inventive preparation showed a limited amount of skin penetration of methylparaben, a skin-irritating substance.

Table 6

Component(%)	Inventive preparation 1	Inventive preparation 2	Comparative preparation
Surfactin sodium salt	3.0	-	-
Dried medium preparation	-	3.0	-
Ethyl alcohol	39.6	39.6	39.6
1,3-Butylene glycol	9.5	9.5	9.5
Castor oil	4.9	4.9	4.9
Tocopherol	1.0	1.0	1.0
Methylparaben	0.2	0.2	0.2
Purified water	Balance	Balance	Balance

Table 7

Test Substance	Concentration of methylparaben
Inventive preparation 1	3.5 ppm
Inventive preparation 2	3.6 ppm
Comparative preparation	7.0 ppm

Example 5

Samples were prepared by dissolving surfactin sodium salt in a concentration of 0 or 0.2% and calcium chloride dihydrate in a calcium concentration of 0, 10, or 20 ppm in each of solvents described below, and each solution was charged in a screw vial and sealed, which was then left to stand at 40℃ for 7 days. After 7 days,

the turbidity of each sample was judged visually. Table 8 shows the results.

Solvent 1: Deionized water

Solvent 2: 7% Ethanol, 93% deionized water

Solvent 3: 7% Ethanol, 5% glycerin, 5% 1,3-butylene glycol, 83% deionized water

Table 8

Composition of Samples	Concentration of calcium(ppm)		
	0	10	20
Solvent 1 + Surfactin 0%	-	-	-
Solvent 2 + Surfactin 0%	-	-	-
Solvent 3 + Surfactin 0%	-	-	-
Solvent 1 + Surfactin 0.2%	-	+	+
Solvent 2 + Surfactin 0.2%	-	+	+
Solvent 3 + Surfactin 0.2%	-	+	+

-: Not turbid
+: Turbid

As will be apparent from the results in Table 8, in solvents containing surfactin sodium salts, turbidity occurs in the presence of calcium.

Example 6

Samples were prepared by dissolving 0.2% surfactin sodium salt and magnesium chloride dihydrate in a magnesium concentration of 0, 10, or 20 ppm in each of

solvents described below, and each solution was charged in a screw vial and sealed, which was then left to stand at 40℃ for 7 days. After 7 days, the turbidity of each sample was judged visually. Table 9 shows the results.

Solvent 1: Deionized water

Solvent 2: 7% Ethanol, 93% deionized water

Solvent 3: 7% Ethanol, 5% glycerin, 5% 1,3-butylene glycol, 83% deionized water

Table 9

Composition of Samples	Concentration of magnesium(ppm)		
	0	10	20
Solvent 1	−	+	+
Solvent 2	−	±	±
Solvent 3	−	±	±

−: Not turbid
±: Slightly turbid
+: Turbid

As will be apparent from the results in Table 9, in solvents containing surfactin sodium salts, turbidity occurs in the presence of magnesium.

Example 7

Samples were prepared by dissolving 0.2% surfactin sodium salt and calcium chloride dihydrate in a calcium concentration of 0.1, 1, or 10 ppm in the solvent described below and further disodium edetate was added

in an amount of 0, 0.0001, 0.001, 0.01, 0.1, 0.2, 1, or 3%, respectively. Then, each solution was charged in a screw vial and sealed, which was then left to stand at 40℃ for 7 days. After 7 days, the turbidity of each sample was judged visually. Table 10 shows the results.

Solvent: 7% Ethanol, 5% glycerin, 5% 1,3-butylene glycol, 83% deionized water.

Table 10

Concentration of sodium edetate(%)	Concentration of calcium(ppm)		
	0.1	1	10
0	−	+	+
0.0001	−	±	+
0.001	−	−	+
0.01	−	−	−
0.1	−	−	−
0.2	−	−	−
1	−	−	−
3	−	−	−

−: Not turbid
±: Slightly turbid
+: Turbid

As will be apparent from the results in Table 10, in solvents containing surfactin sodium salt and calcium, addition of disodium edetate prevented turbidity from occurring.

Example 8

Samples were prepared by dissolving 0.2% surfactin sodium salt and calcium chloride dihydrate in a calcium concentration of 0.1, 1, or 10 ppm in the solvent described below and further sodium citrate was added in an amount of 0, 0.0001, 0.001, 0.01, 0.1, 0.2, 1, or 3%, respectively. Then, each solution was charged in a screw vial and sealed, which was then left to stand at 40℃ for 7 days. After 7 days, the turbidity of each sample was judged visually. Table 11 shows the results.

Solvent: 7% Ethanol, 5% glycerin, 5% 1,3-butylene glycol, 83% deionized water.

Table 11

Concentration of sodium citrate(%)	Concentration of calcium(ppm)		
	0.1	1	10
0	−	+	+
0.0001	−	±	+
0.001	−	−	+
0.01	−	−	−
0.1	−	−	−
0.2	−	−	−
1	−	−	−
3	−	−	−

−: Not turbid
±: Slightly turbid
+: Turbid

As will be apparent from the results in Table 11, in solvents containing surfactin sodium salt and calcium, addition of sodium citrate is effective in inhibiting the occurrence of turbidity, similarly to disodium edetate.

Example 9

Milky lotions having the composition shown in Table 12 were prepared and were evaluated for their skin irritation. In the same manner as in Example 1, a skin model was exposed to each of the prepared test substances (milky lotions described in Table 12) for 1 hour. Thereafter, the test substance was washed and incubated in the above-described medium for 16 hours. After the incubation, a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added, and pigments were extracted with isopropanol rendered acidic with hydrochloric acid, followed by measurement of absorbance and calculation of cytotoxicity. The cytotoxicity was calculated according to the equation described in Example 1. Table 13 shows cytotoxicity of the test substances.

Table 12

Component(%)	Inventive preparation 1	Inventive preparation 2	Inventive preparation 3	Inventive preparation 4	Comparative preparation 1	Comparative preparation 2
Surfactin sodium salt	3.0	-	3.0	-	3.0	-
Dried medium preparation	-	3.0	-	3.0	-	3.0
Disodium edetate	0.01	0.01	-	-	-	-
Sodium citrate	-	-	0.01	0.01	-	-
Avogado oil	11.0	11.0	11.0	11.0	11.0	11.0
Behenyl alcohol	0.6	0.6	0.6	0.6	0.6	0.6
Stearic acid	0.4	0.4	0.4	0.4	0.4	0.4
1,3-Butylene glycol	10.1	10.1	10.1	10.1	10.1	10.1
Perfume	0.4	0.4	0.4	0.4	0.4	0.4
Purified water	Balance	Balance	Balance	Balance	Balance	Balance

Table13

Test Substance	Cytotoxicity
Inventive preparation 1	0.0 %
Inventive preparation 2	0.0 %
Inventive preparation 3	0.0 %
Inventive preparation 4	0.0 %
Comparative preparation 1	0.0 %
Comparative preparation 2	0.0 %

As will be apparnt from the results shown in Table 13, the inventive preparation like the comparative preparations had very low sin irritation and the addition of disodium edetate or sodium citrate did not affect on the low irritation.

Example 10

One (donor side) of two chambers separated by a hairless mouse skin was filled with a cosmetic having the composition described in Table 14 and the other (receiver side) was filled with a phosphate buffer solution (pH 7). After 5 hours, the concentration of methylparaben on the receiver side was measured. Table 15 shows the results.

Table 14

Component(%)	Inventive Preparation				Comparative Preparation				
	1	2	3	4	1	2	3	4	5
Surfactin sodium salt	3.0	-	3.0	-	-	-	3.0	-	-
Dried medium preparation	-	3.0	-	3.0	-	-	-	3.0	-
Disodium edetate	0.01	0.01	-	-	0.01	-	-	-	-
Sodium citrate	-	-	0.01	0.01	-	0.01	-	-	-
Ethyl alcohol	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6
1,3-Butylene glycol	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Castor oil	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
α-Tocopherol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Methylparaben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Purified water	Balance	Balance	Balance	Balance	Balance	Balance	Balance	Balance	Balance

Table 15

Test Substance	Concentration of methylparaben
Inventive preparation 1	3.6 ppm
Inventive preparation 2	3.7 ppm
Inventive preparation 3	3.5 ppm
Inventive preparation 4	3.6 ppm
Comparative preparation 1	7.2 ppm
Comparative preparation 2	7.1 ppm
Comparative preparation 3	3.5 ppm
Comparative preparation 4	3.6 ppm
Comparative preparation 5	7.0 ppm

As will be apparent from the results, the inventive preparation like the comparative preparations 3 and 4 suppressed the skin penetration of methylparaben, a skin-irritating substance. Therefore, it revealed that the addition of disodium edetate or sodium citrate did not affect on the effect of reducing the irritation of skin-irritating substances.

Formulation Examples

In the following formulation examples of external preparation for skin, APM and APS indicate magnesium ascorbic acid 2-phosphate and sodium ascorbic acid 2-phosphate. Also, "%" means "wt%".

Formulation Example 1: Beauty wash

The following components were dissolved with heating at 50℃ and cooled with stirring until the temperature reached 30℃ when the stirring was stopped and left to stand to prepare a beauty wash.

Ethyl alcohol	39.6%
1,3-Butylene glycol	9.5%
Castor oil	4.9%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
Methylparaben	0.2%
Surfactin	1.0%
Purified water	balance

Formulation Example 2: Milky lotion

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a milky lotion.

Avogado oil	11.0%
Behenyl alcohol	0.6%
Stearic acid	0.4%
Glycerin fatty acid ester	0.9%
Polyoxyethylene sorbitan fatty acid ester	1.1%

Polyoxyethylene alkyl ether	0.4%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
1,3-Butylene glycol	10.1%
Methylparaben	0.2%
Perfume	0.4%
Surfactin	0.5%
Purified water	balance

Formulation Example 3: Cream

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a cream.

Squalane	11.1%
Stearic acid	7.8%
Stearyl alcohol	6.0%
Beeswax	1.9%
Propylene glycol monostearate	3.1%
Polyoxyethylene cetyl ether	1.1%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
1,3-Butylene glycol	10.1%

Methylparaben	0.2%
Perfume	0.4%
Surfactin	0.5%
Purified water	balance

Formulation Example 4: Pack

The following components were swelled with heating at 50℃ and uniformly mixed with stirring. This was cooled with stirring and the stirring was stopped at 30℃, and the mixture was left to stand to prepare a pack.

Polyvinyl alcohol	14.5%
Sodium carboxymethylcellulose	4.8%
1,3-Butylene glycol	2.9%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
Ethyl alcohol	10.0%
Methylparaben	0.1%
Surfactin	0.5%
Purified water	balance

Formulation Example 5: Lipstick

A red pigment was dispersed in castor oil using a roll mill to prepare a dispersion (A). To the dispersion were dissolved with heating other blending components in the following proportions and mixed well. The mixture

was filtered and cast in a mold at a high temperature and cooled. The molded composition was charged in a vessel to prepare a lipstick.

Castor oil	45.3%
Hexadecyl alcohol	25.2%
Lanoline	3.9
Beeswax	4.8%
Ozocerite	3.4%
Candelilla wax	6.2%
Carnauba wax	2.1%
Methylparaben	0.1%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
Titanium oxide	2.1%
Red pigment	4.8%
Perfume	0.1%
Surfactin	0.1%
Moisture	balance

Formulation Example 6: Beauty wash

The following components were dissolved with heating at 50℃ and cooled with stirring. The stirring was stopped at 30℃, and the mixture was left to stand to prepare a beauty wash.

Ethyl alcohol	39.6%

1,3-Butylene glycol	9.5%
Castor oil	4.9%
α-Tocopherol	1.0%
APM or APS	3.0%
Kojic acid	1.0%
Placenta extract	1.0%
Albutin	1.0%
Citric acid	0.5%
Tartaric acid	0.5%
Malic acid	0.5%
NaOH	(s. a. to make weakly alkaline pH)
Methylparaben	0.2%
Surfactin	0.5%
Purified water	balance

Formulation Example 7: Beauty wash

The following components were dissolved with heating at 50℃ and cooled with stirring. The stirring was stopped at 30℃, and the mixture was left to stand to prepare a beauty wash.

Ethyl alcohol	39.6%
1,3-Butylene glycol	9.5%
Castor oil	4.9%
α-Tocopherol	1.0%
APM or APS	3.0%
Kojic acid	1.0%

Placenta extract	1.0%
Albutin	1.0%
Citric acid	0.5%
Tartaric acid	0.5%
Malic acid	0.5%
EDTA 2Na	1.0%
NaOH	(s. a. to make weakly alkaline pH)
Methylparaben	0.2%
Surfactin	0.5%
Purified water	balance

Formulation Example 8: Beauty wash

The following components were dissolved with heating at 50℃ and cooled with stirring. The stirring was stopped at 30℃, and the mixture was left to stand to prepare a beauty wash.

Ethyl alcohol	39.6%
1,3-Butylene glycol	9.5%
Castor oil	4.9%
APM or APS	3.0%
Methylparaben	0.2%
Surfactin	0.5%
Purified water	balance

Formulation Example 9: Milky lotion

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a milky lotion.

Avogado oil	11.0%
Behenyl alcohol	0.6%
Stearic acid	0.4%
Glycerin fatty acid ester	0.9%
Polyoxyethylene sorbitan fatty acid ester	1.1%
Polyoxyethylene alkyl ether	0.4%
APM or APS	3.0%
1,3-Butylene glycol	10.1%
Methylparaben	0.2%
Perfume	0.4%
Surfactin	0.5%
Purified water	balance

Formulation Example 10: Cream

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a cream.

Squalane	11.1%

Stearic acid	7.8%
Stearyl alcohol	6.0%
Beeswax	1.9%
Propylene glycol monostearate	3.1%
Polyoxyethylene cetyl ether	1.1%
APM or APS	3.0%
1,3-Butylene glycol	11.9%
Methylparaben	0.2%
Perfume	0.4%
Surfactin	1.0%
Purified water	balance

Formulation Example 11: Pack

The following components were swelled with heating at 50℃ and uniformly mixed with stirring. This was cooled with stirring and the stirring was stopped at 30℃, and the mixture was left to stand to prepare a pack.

Polyvinyl alcohol	14.5%
Sodium carboxymethylcellulose	4.8%
1,3-Butylene glycol	2.9%
APM or APS	3.0%
Ethyl alcohol	10.0%
Ethylparaben	0.1%
Surfactin	0.1%
Purified water	balance

Formulation Example 12: Lipstick

A red pigment was dispersed in castor oil using a roll mill to prepare a dispersion (A). To the dispersion were dissolved with heating other blending components in the following proportions and mixed well. The mixture was filtered and cast in a mold at a high temperature and cooled. The molded composition was charged in a vessel to prepare a lipstick.

Castor oil	45.3%
Hexadecyl alcohol	25.2%
Lanoline	3.9%
Beeswax	4.8%
Ozocerite	3.4%
Candelilla wax	6.2%
Carnauba wax	2.1%
Methylparaben	0.1%
APM or APS	3.0%
Titanium oxide	2.1%
Red pigment	4.8%
Perfume	0.1%
Surfactin	0.1%
Moisture	balance

Formulation Example 13: Foundation

The following components were mixed at 80℃ with stirring and then left to cool to prepare a foundation.

Liquid paraffin	23.5%
Isopropyl palmitate	14.3%
Lanoline alcohol	1.8%
Lanoline acetate	2.9%
Microcrystalline wax	6.5%
Ozocerite	7.7%
Candelilla wax	0.4%
Methylparaben	0.1%
APM or APS	3.0%
Titanium oxide	14.5%
Kaolin	13.9%
Talc	5.7%
Coloring pigment	3.9%
Perfume	0.5%
Surfactin	0.1%
Moisture	balance

Formulation Example 14: Dentifrice

The following compositions were swelled with heating, mixed well, and then left to stand to prepare a dentifrice composition.

Calcium secondary phosphate dihydrate	45.5%
Sodium carboxymethylcellulose	0.5%
Carrageenan	0.5%
Glycerin	9.8%

Sorbitol	9.7%
Sodium saccharinate	0.1%
Surfactin	2.0%
Sodium chloride	2.1%
α-Tocopherol	0.4%
APM or APS	3.0%
Antiseptic	0.1%
Perfume	0.5%
Purified water	balance

Formulation Example 15: Gargle

The following components were uniformly mixed at ambient temperature to prepare a gargle.

Ethyl alcohol	34.6%
Glycerin	14.5%
α-Tocopherol	0.4%
APM or APS	3.0%
Surfactin	0.1%
Perfume	0.5%
Purified water	balance

Formulation Example 16: Hair tonic

The following components were uniformly mixed at ambient temperature to prepare a hair tonic.

Ethyl alcohol	63.0%
Castor oil	4.3%

Resorcinol	0.7%
Methylparaben	0.1%
Capsicum tincture	0.4%
α-Tocopherol	0.4%
APM or APS	3.0%
Surfactin	0.2%
Purified water	balance

Formulation Example 17: Shampoo

The following components were dissolved by heating at 70℃ and mixed with stirring. Then this was cooled with stirring to 40℃ and left to stand to prepare a shampoo.

Triethanolamine laurylsulfate	15.0%
Diethanolamide laurate	3.3%
Triethanolamine polyacrylate	0.3%
Zinc pyridinium-1-thiol-N-oxide	1.1%
APM or APS	3.0%
Surfactin	1.0%
Pigment	minute amount
Perfume	0.5%
Purified water	balance

Formulation Example 18: Rinse

The following components were dissolved by heating at 80℃ and mixed with stirring. Then this was cooled

with stirring to 40℃ and left to stand to prepare a rinse.

Stearyldimethylbenzylammonium chloride	1.4%
Stearyl alcohol	0.6%
Glyceryl monostearate	1.5%
Sodium chloride	0.2%
APM or APS	3.0%
Surfactin	0.1%
Purified water	balance

Formulation Example 19: Bath agent

The following components were uniformly mixed at ambient temperature to prepare a bath agent.

Sodium hydrogen carbonate	35.5%
Citric acid	37.1%
Polyethylene glycol	2.1%
Magnesium chloride	1.1%
α-Tocopherol	0.5%
APM or APS	24.0%
Surfactin	1.0%
Pigment	minute amount
Perfume	2.0%

Formulation Example 20: Beauty wash

The following components were dissolved with heating at 50℃ and cooled with stirring. The stirring was stopped at 30℃, and the mixture was left to stand to prepare a beauty wash.

Ethyl alcohol	39.6%
1,3-Butylene glycol	9.5%
Castor oil	4.9%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
Methylparaben	0.2%
Surfactin	1.0%
Disodium edetate	0.01%
Purified water	balance

Formulation Example 21: Milky lotion

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a milky lotion.

Avogado oil	11.0%
Behenyl alcohol	0.6%
Stearic acid	0.4%
Glycerin fatty acid ester	0.9%
Polyoxyethylene sorbitan fatty	

acid ester	1.1%
Polyoxyethylene alkyl ether	0.4%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
1,3-Butylene glycol	10.1%
Methylparaben	0.2%
Perfume	0.4%
Surfactin	0.5%
Disodium edetate	0.01%
Purified water	balance

Formulation Example 22: Cream

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a cream.

Squalane	11.1%
Stearic acid	7.8%
Stearyl alcohol	6.0%
Beeswax	1.9%
Propylene glycol monostearate	3.1%
Polyoxyethylene cetyl ether	1.1%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%

Sodium ascorbic 2-phosphate	1.0%
1,3-Butylene glycol	10.1%
Methylparaben	0.2%
Perfume	0.4%
Surfactin	0.5%
Sodium citrate	0.01%
Purified water	balance

Formulation Example 23: Pack

The following components were swelled with heating at 50℃ and uniformly mixed with stirring. This was cooled with stirring and the stirring was stopped at 30℃, and the mixture was left to stand to prepare a pack.

Polyvinyl alcohol	14.5%
Sodium carboxymethylcellulose	4.8%
1,3-Butylene glycol	2.9%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
Ethyl alcohol	10.0%
Methylparaben	0.1%
Surfactin	0.5%
Sodium citrate	0.01%
Purified water	balance

Formulation Example 24: Lipstick

A red pigment was dispersed in castor oil using a roll mill to prepare a dispersion (A). To the dispersion were dissolved with heating other blending components in the following proportions and mixed well. The mixture was filtered and cast in a mold at a high temperature and cooled. The molded composition was charged in a vessel to prepare a lipstick.

Castor oil	45.3%
Hexadecyl alcohol	25.2%
Lanoline	3.9%
Beeswax	4.8%
Ozocerite	3.4%
Candelilla wax	6.2%
Carnauba wax	2.1%
Methylparaben	0.1%
α-Tocopherol	1.0%
Magnesium ascorbic 2-phosphate	1.0%
Sodium ascorbic 2-phosphate	1.0%
Titanium oxide	2.1%
Red pigment	4.8%
Perfume	0.1%
Surfactin	0.1%
Disodium edetate	0.005%
Moisture	balance

Formulation Example 25: Beauty wash

The following components were dissolved with heating at 50℃ and cooled with stirring. The stirring was stopped at 30℃, and the mixture was left to stand to prepare a beauty wash.

Ethyl alcohol	39.6%
1,3-Butylene glycol	9.5%
Castor oil	4.9%
APM or APS	3.0%
Sodium citrate	0.1%
Methylparaben	0.2%
Surfactin	0.5%
Purified water	balance

Formulation Example 26: Milky lotion

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a milky lotion.

Avogado oil	11.0%
Behenyl alcohol	0.6%
Stearic acid	0.4%
Glycerin fatty acid ester	0.9%
Polyoxyethylene sorbitan fatty acid ester	1.1%
Polyoxyethylene alkyl ether	0.4%

APM or APS	3.0%
1,3-Butylene glycol	10.1%
Disodium edetate	0.01%
Methylparaben	0.2%
Perfume	0.4%
Surfactin	0.5%
Purified water	balance

Formulation Example 27: Cream

The following components were dissolved with heating at 80℃ with stirring to emulsify. This was cooled with stirring and the stirring was stopped at 40℃, and the mixture was left to stand to prepare a cream.

Squalane	11.1%
Stearic acid	7.8%
Stearyl alcohol	6.0%
Beeswax	1.9%
Propylene glycol monostearate	3.1%
Polyoxyethylene cetyl ether	1.1%
APM or APS	3.0%
1,3-Butylene glycol	11.9%
Disodium edetate	0.01%
Methylparaben	0.2%
Perfume	0.4%
Surfactin	1.0%

Purified water	balance

Formulation Example 28: Pack

The following components were swelled with heating at 50℃ and uniformly mixed with stirring. This was cooled with stirring and the stirring was stopped at 30℃, and the mixture was left to stand to prepare a pack.

Polyvinyl alcohol	14.5%
Sodium carboxymethylcellulose	4.8%
1,3-Butylene glycol	2.9%
APM or APS	3.0%
Ethyl alcohol	10.0%
Disodium edetate	0.001%
Ethylparaben	0.1%
Surfactin	0.1%
Purified water	balance

Formulation Example 29: Lipstick

A red pigment was dispersed in castor oil using a roll mill to prepare a dispersion (A). To the dispersion were dissolved with heating other blending components in the following proportions and mixed well. The mixture was filtered and cast in a mold at a high temperature and cooled. The molded composition was charged in a vessel to prepare a lipstick.

Castor oil	45.3%

Hexadecyl alcohol	25.2%
Lanoline	3.9%
Beeswax	4.8%
Ozocerite	3.4%
Candelilla wax	6.2%
Carnauba wax	2.1%
Methylparaben	0.1%
APM or APS	3.0%
Titanium oxide	2.1%
Red pigment	4.8%
Perfume	0.1%
Edetic acid	0.001%
Surfactin	0.1%
Moisture	balance

Formulation Example 30: Foundation

The following components were mixed at 80℃ with stirring and then left to cool to prepare a foundation.

Liquid paraffin	23.5%
Isopropyl palmitate	14.3%
Lanoline alcohol	1.8%
Lanoline acetate	2.9%
Microcrystalline wax	6.5%
Ozocerite	7.7%
Candelilla wax	0.4%
Methylparaben	0.1%

APM or APS	3.0%
Titanium oxide	14.5%
Kaolin	13.9%
Talc	5.7%
Coloring pigment	3.9%
Perfume	0.5%
Disodium edetate	0.001%
Surfactin	0.1%
Moisture	balance

Formulation Example 31: Dentifrice

The following compositions were swelled with heating, mixed well, and then left to stand to prepare a dentifrice composition.

Calcium secondary phosphate dihydrate	45.5%
Sodium carboxymethylcellulose	0.5%
Carrageenan	0.5%
Glycerin	9.8%
Sorbitol	9.7%
Sodium saccharinate	0.1%
Surfactin	2.0%
Sodium chloride	2.1%
α-Tocopherol	0.4%
APM or APS	3.0%
Disodium edetate	0.1%

Antiseptic	0.1%
Perfume	0.5%
Purified water	balance

Formulation Example 32: Gargle

The following components were uniformly mixed at ambient temperature to prepare a gargle.

Ethyl alcohol	34.6%
Glycerin	14.5%
α-Tocopherol	0.4%
APM or APS	3.0%
Sodium citrate	0.01%
Surfactin	0.1%
Perfume	0.5%
Purified water	balance

Formulation Example 33: Hair tonic

The following components were uniformly mixed at ambient temperature to prepare a hair tonic.

Ethyl alcohol	63.0%
Castor oil	4.3%
Resorcinol	0.7%
Methylparaben	0.1%
Capsicum tincture	0.4%
α-Tocopherol	0.4%
APM or APS	3.0%

Disodium edetate	0.01%
Surfactin	0.2%
Purified water	balance

Formulation Example 34: Shampoo

The following components were dissolved by heating at 70℃ and mixed with stirring. Then this was cooled with stirring to 40℃ and left to stand to prepare a shampoo.

Triethanolamine laurylsulfate	15.0%
Diethanolamide laurate	3.3%
Triethanolamine polyacrylate	0.3%
Zinc pyridinium-1-thiol-N-oxide	1.1%
APM or APS	3.0%
Disodium edetate	0.05%
Surfactin	1.0%
Pigment	minute amount
Perfume	0.5%
Purified water	balance

Formulation Example 35: Rinse

The following components were dissolved by heating at 80℃ and mixed with stirring. Then this was cooled with stirring to 40℃ and left to stand to prepare a rinse.

Stearyldimethylbenzylammonium

chloride	1.4%
Stearyl alcohol	0.6%
Glyceryl monostearate	1.5%
Sodium chloride	0.2%
APM or APS	3.0%
Disodium edetate	0.001%
Surfactin	0.1%
Purified water	balance

Formulation Example 36: Bath agent

The following components were uniformly mixed at ambient temperature to prepare a bath agent.

Sodium hydrogen carbonate	35.5%
Citric acid	37.1%
Polyethylene glycol	2.1%
Magnesium chloride	1.1%
α-Tocopherol	0.5%
APM or APS	24.0%
Disodium edetate	1.0%
Surfactin	0.2%
Pigment	minute amount
Perfume	2.0%

INDUSTRIAL APPLICABILITY

The surfactant for use in external preparations for skin of the present invention has low skin penetration and low skin irritation. Therefore, they can be applied to cosmetics. Cosmetics usually contain skin-irritating substances and addition of the surfactant of the present invention can reduce the irritation of the irritating substances.

Surfactin, a typical example of the surfactant for use in external preparations for skin, can be produced by utilizing microbes so that it is advantageous from the viewpoint of production method.

Further, the external preparation for skin containing a sequestering agent according to the present invention can suppress the generation of turbidity in cosmetics attributable to the surfactant for use in external preparations for skin and alkaline earth metal and can retain transparency of cosmetics. Therefore, it is very advantageous in its application to transparent cosmetics.

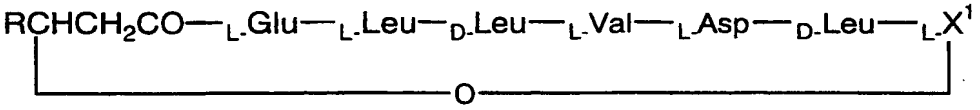
CLAIMS

1. A surfactant for use in external preparations for skin comprising a compound derived from a prokaryote.

2. The surfactant for use in external preparations for skin as claimed in Claim 1, wherein the prokaryote is a *Bacillus* microbe.

3. The surfactant for use in external preparations for skin as claimed in Claim 1, wherein the compound derived from prokaryote is a lipopeptide compound or its salts.

4. The surfactant for use in external preparations for skin as claimed in Claim 3, wherein the lipopeptide compound is at least one compound represented by formula (2) below



(wherein X^1 is an amino acid selected from the group consisting of leucine, isoleucine, valine, glycine, serine, alanine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, cystein, methionine, phenylalanine, tyrosine, tryptophan, histidine, proline, 4-hydroxyproline, and

homoserine, and R has 9 to 13 carbon atoms and is a n-alkyl group, an isoalkyl group, or an anteisoalkyl group).

5. The surfactant for use in external preparations for skin as claimed in Claim 4, wherein X^1 is leucine, isoleucine or valine.

6. The surfactant for use in external preparations for skin as claimed in Claim 3, wherein the lipopeptide compound is plipastatin, arthrofactin, iturin, or serrawettin.

7. The surfactant for use in external preparations for skin as claimed in any one of Claims 1 to 6, wherein the surfactant has an effect of inhibiting the skin penetration of a skin-irritating substance.

8. The surfactant for use in external preparations for skin as claimed in any one of Claims 1 to 6, wherein the surfactant has an effect of inhibiting the skin penetration of a skin-irritating substance and reduces irritation of a skin-irritating substance.

9. The surfactant for use in external preparations for skin as claimed in Claim 8, wherein the skin-irritating substance is an antiseptic.

10. The surfactant for use in external preparations for skin as claimed in Claim 9, wherein the antiseptic is a paraben compound.

11. An external preparation for skin comprising a surfactant for use in external preparations as claimed in any one of Claims 1 to 10.

12. The external preparation for skin as claimed in Claim 11, wherein the surfactant for use in external preparations for skin is in a content of 0.01 to 30 wt%.

13. The external preparation for skin as claimed in Claim 11 or 12, further comprising a sequestering agent.

14. The external preparation for skin as claimed in Claim 13, wherein the surfactant for use in external preparations for skin is in a content of 0.01 to 30 wt% and the sequestering agent is in a content of 0.0001 to 30 wt%.

15. A cosmetic comprising an external preparation for skin as claimed in any one of Claims 11 to 14.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/JP 99/02858

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 600 526 A (GALLOT BERNARD ET AL) 15 July 1986 (1986-07-15) column 7, line 61 - line 65; claims 1-15	1,3
A	FR 2 668 365 A (SEDERMA SA) 30 April 1992 (1992-04-30)	
A	CHEMICAL ABSTRACTS, vol. 128, no. 20, 18 May 1998 (1998-05-18) Columbus, Ohio, US; abstract no. 248380, page 1004; XP002115063 abstract & JP 10 059832 A (LION CORP) 3 March 1998 (1998-03-03)	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 1999	24/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stienon, P

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INTERNATIONAL SEARCH REPORT

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Interr nal Application No
PCT/JP 99/02858

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